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METHODS FOR MARKING INSECTS: Current Techniques and Future Prospects

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■ **Abstract** Tracking the movement of insects in their natural habitat is essential for understanding their basic biology, demography, and ethology. A wide variety of markers have been used to assess insect population dynamics, dispersal, territoriality, feeding behavior, trophic-level interactions, and other ecological interactions. The ideal marker should persist without inhibiting the insect's "normal" biology. Furthermore, the marker should be environmentally safe, cost–effective, and easy to use. In this article, we review the current state of knowledge regarding insect marking, document the advantages and limitations of each marking technique, and discuss advances made in marking insects over the past decade.

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INTRODUCTION

A reliable method for marking animals is often a key component in studies of animal biology, ethology, and demography. Animal marking dates back to 218 B.C., when ornithologists distinguished ownership of birds by banding (59). Insect marking for scientific studies began around 1920, when researchers used paints, dyes, and stains in studies of insect population dynamics (54, 67). Hundreds if not thousands of studies that required some way to label insects have been conducted, but the search for a universal marker has proven to be challenging. Owing to the overwhelming amount of literature on insect marking, it is virtually impossible to write an all-inclusive review. Instead, we provide readers with broad examples of many of the insect-marking techniques that have been used, and we provide an overview of the advantages, disadvantages, and limitations of each technique. Finally, we highlight advances made in marking insects over the past decade.

CHARACTERISTICS OF EFFECTIVE MARKERS

A wide variety of materials and methods have been used to mark animals for biological research. Vertebrate biologists often mark their test subjects with bands, brands, tattoos, tags, notches, paints, and radiolabels (17, 202). Unfortunately, most vertebrate-marking techniques are not practical for marking insects because they are cumbersome, heavy, and/or costly (193). As a result, entomologists are often challenged to develop unique methods for marking insects.

Investigators should not assume that any given marking procedure is applicable for their research. In many of the studies described below, an ideal marker for one insect species is a useless marker for other insects (205). Preliminary studies should be done before conducting any investigation in which a marker is going to be used to identify an insect, to ensure that it is retained on the insect for a sufficient period of time and that the marker does not adversely affect the insect. An ideal marking material is durable, inexpensive, nontoxic (to the insect and the environment), easily applied, and clearly identifiable. Furthermore, the marker should not hinder or irritate the insect or affect its normal behavior, growth, reproduction, or life span.

The method of choice for applying markers depends on the insect being marked, the environment that the insect will encounter, and the nature of the experiment. Insects can be marked individually or in large groups. Individual marks, usually in the form of a painted label or a physical tag, permit the identification of a specific

individual in a population. Mass marking, usually in the form of an application of dust, paint, or dye, permits the identification of a group of insects within a larger population. In addition to individual-marking and group-marking techniques, self-marking techniques have been developed in which insects mark themselves by contacting marking materials that are natural to their environment (e.g. pollen) or materials that have been strategically placed in their environment by researchers (e.g. bait formulations or substances at the entrances of nests or hives).

TYPES OF STUDIES THAT REQUIRE A MARKER

The selection of a specific marker depends on the type of study that the researcher is planning. We describe marking procedures for two broad categories of research—mark-release-recapture (MRR) and mark-capture (177).

Mark-Release-Recapture

For studies using MRR techniques, the researcher collects insects either from laboratory colonies or from the field. The collected insects are then marked, released into the field, and recaptured at given time and distance intervals after their release. The recaptured insects are then checked for the presence of the marker to distinguish them from unmarked insects. All of the marking materials described below can be used for MRR studies; however, some of the marking techniques are more practical than others. MRR studies are particularly useful for mass marking known populations of insects for dispersal studies.

Mark-Capture

For studies using mark-capture techniques, the researcher applies the marker to the insects directly in the field (177). Many of the marking procedures described below are obviously not practical for mark-capture studies (e.g. tags or mutilation). The most useful markers for mark-capture studies are those materials that are inexpensive and can be easily applied via broadcast application to the insects' natural habitat or those materials that can be added to insect bait.

MATERIALS AND TECHNIQUES USED TO MARK INSECTS

Tags and Related Markers

Biologists have used a wide variety of techniques to externally affix tags, labels, and bands for marking individual animals. Tags are frequently used to mark individual mammals, reptiles, amphibians, fish, and birds for conservation, dispersal,

and population ecology studies (4, 17, 60). In some situations, tags are useful for marking insects. However, most tags are too large and heavy for use on insects.

Early researchers glued numbered pieces of paper or cellophane on insects. Small plastic tags made from photographic film have been used to mark screwworms [*Cochliomyia hominivorax* (181)]. These tags were made by photographically reducing a series of two-alphanumeric codes, printed in a continuous, nonrepeating series, onto the film.

Honeybees (*Apis mellifera*) are frequently marked with tags. Sequentially numbered bee tags are commercially available in a variety of colors that can simply be glued onto the thorax of bees. Ferrous-metal numbered tags have been attached to bees in the field. As the bees return to their hive, they are recaptured with magnets strategically placed at the hive entrance (65). This metal-tagging procedure was useful for examining the flight range and foraging activity of individual honeybees.

Individual members of an ant colony have been tagged by using an elaborate wire-banding system. Wire bands either 30- or 60-μm in diameter were tied to various body regions of ants (e.g. petiole joint, second petiole joint, gaster, etc), using several different types of knots and knot orientations. Hundreds of ants could be tagged individually based on the various combinations of wire diameter, placement, and knot (174).

The major advantages to using tags to mark insects are that they are inexpensive and they can be used to identify insects on an individual basis for MRR studies. Tags are most useful for long-term studies in which enamel paints would not be retained. The size, shape, and placement of tags must not restrict the insect's movement or interfere with behavior (64, 193).

A major disadvantage of using tags to mark insects is that the application of individual tags is tedious and time-consuming. These drawbacks make tagging procedures impractical for mass marking insects. Moreover, the physical nature of tags limits their application to relatively large insect species.

Mutilation Marking

Mutilation marking has been used to mark animals for over a century (203). Mutilation involves clipping, punching, notching, or etching a distinctive mark on an animal so that it can be distinguished easily from its counterparts. These techniques are usually reserved for marking large animals such as fish, amphibians, reptiles, cattle, and birds (17, 203). Mutilation is rarely used for marking insects, and, for the most part, it is useful only for marking large or heavily sclerotized insects, such as beetles, or insects with large wings, such as butterflies, dragonflies, and grasshoppers (21, 64, 215).

Lepidopterans are sometimes tagged by clipping the wings of adults (175) or the prolegs of larvae (224). For example, gypsy moth (*Lymantria dispar*) larvae were marked by removing different prolegs or numbers of prolegs to study their movement, survival, and population dynamics (224). A "notching" technique was used to mark many different orthopteran species (64). The technique consisted

of combining the amputation of the tegmina with notching various portions of the fore, hind, and lateral margins of the pronotum. Various arrangements of the notches, in combination with full or partial amputation of the tegmina, yielded a large number of distinctive marks. This notching technique was not harmful to the insects and was persistent through the later life stages of the insect, as the molting insects retained their distinctive marks.

Distinctive marks have also been etched or punctured onto the elytra of adult beetles using insect pins (21, 215). Some beetles produce a melanized dark spot when they are punctured (Figure 1). This characteristic allows for the marking of beetles by simply puncturing the elytra with the tip of a pin. Studies have shown that puncturing and etching do not produce any noticeable side effects on beetle life span and require less handling time and manipulation than tagging procedures described above (31, 91).

The mutilation technique used must not adversely affect the insect's normal behavior or dispersal ability. Mutilation techniques also require extensive handling of individual insects, which can make the procedure tedious, time-consuming, and harmful to the insect. For some species, such as large beetles, the mark can be applied by simply holding the insect between the fingers and puncturing or etching the mark on an insect with a minuten pin (193, 215). However, in most cases, the insect must be anaesthetized with CO_2, chilled, or held down with some sort of device relying on vacuum suction or an adhesive. Most authors using mutilation marks have not reported any adverse affects on their test insects (64, 215).

The major advantages of marking by mutilation are that the marks are usually persistent and that they can be readily and accurately recognized in the field without the aid of any specialized equipment. The major limitation of mutilation is that it can be tedious and time-consuming if a large number of insects require individual marks. Furthermore, mutilation is applicable to only a small number of insect species.

Paint and Ink Marking

Paints and inks were among the first materials used to mark insects, and they are still among the most commonly used materials for marking individual and groups of insects (193, 229).

Marking Individual Insects with Paints and Inks Paints and inks are applied to individual insects with toothpicks, insect pins, fine-tipped pens, or fine-haired brushes (193). Recently, red imported fire ants [*Solenopsis invicta* (=*wagneri*)], were marked with ballpoint paint pens (230).

Paints and inks have been applied to individual insects with various degrees of success. Wineriter & Walker (229) tested 26 paints and inks on two cricket and one beetle species and found that non-water-soluble paints had the greatest durability on their surfaces. An ideal paint or ink marker should be durable, nontoxic, easy

to apply, quick drying, lightweight, available in several highly visible colors, and resistant to peeling or chipping (144, 222).

Individual insects were marked with paint or ink using simple numbering systems (64, 93, 143, 185, 193) or elaborate coding systems (25, 158, 178, 225). For example, late-instar larvae of the nymphalid *Euphydryas* (= *Occidryas*) *editha bayensis* were marked by strategically placing three dots of paint on each side of the larvae. White & Singer (226) assigned numeric codes to each larval segment, enabling each larva to be coded with any 1 of 50 different combinations. Similarly, Humphry & Linit (113) used a six-dot binary-coding system to uniquely mark 63 individual beetles.

The major advantages of using paint or ink to mark individual insects are that these techniques are inexpensive and the specimens can be nondestructively sampled and resampled over the course of a long-term study (143). Several authors report that individual markers are durable and easy to apply (93, 113, 144, 158).

The major disadvantages of using paints and inks for marking individual insects are that the application of the marker is often tedious and time-consuming (229). Also, the marker or the solvent is often toxic to insects (see references in 193), and the marker is usually restricted to a single life stage because immature insects lose the mark when they molt. Moreover, paints and inks are usually used only on large insects because of the logistical difficulties of applying a mark to small insects.

Marking Groups of Insects with Paints and Inks A variety of paints and inks can be applied topically to large batches of insects for MRR studies using various spraying devices including hand atomizers and spray guns (50, 170, 231). Mass marking insects with paints or inks is easy, rapid, and inexpensive. Moreover, the marker is usually recognizable on recaptured insects without microscopic examination. Most paints are too thick and sticky to be sprayed directly on insects. Paints are often diluted with acetone or alcohol before application. Researchers must be sure the paint and solvent are nontoxic and do not alter insect behavior. Topical sprays may be destructive to small and delicate insects; therefore, these are usually reserved for large, sturdy insects.

Dust Marking

Dusts (also known as "powders") have been used to mark insects for >75 years (48). To date, they are probably the most commonly used materials for externally marking a variety of insects (185). Various kinds of dusts have been used to label insects (169, 185, 193, 199, 208). An invisible green fluorescent dust used in crime detection was among the first dusts used to mark insects (169, 208). This dust is invisible under normal light, but it is easily detected under UV light. The most common commercial dust used to mark insects is Day-Glo (Day-Glo Color Corp., Cleveland, OH), an affordable fluorescent dust that is available in a wide variety of bright colors. Day-Glo is visible to the naked eye; however, the detection of Day-Glo on insects can be enhanced under UV light (18, 199).

Dusts are most useful for marking large insects or insects with hairy surfaces. Sometimes adjuvants, such as flour, sand, or gum arabic, are mixed with dusts to provide better adhesion of the dust particles (18, 27, 154, 176, 191). Dusts are usually applied to sturdy insects by putting them in a container with a given amount of dust and shaking the container. Often the container is as simple as a paper or plastic bag (153, 199). Tumbling devices have been used to mark adult boll weevils and fruit fly pupae (183, 208), but most shaking and tumbling procedures are not practical for dusting small and delicate insect species because they cause immediate high mortality and place too much dust on the insect (147). Too much dust causes undesirable side effects such as further mortality, decreased mobility, and interference with sensory organs (40). As a result, many innovative devices are available for applying minute quantities of dusts to insects (186). Mosquitoes may be marked with dusts by "puffing" them, using an insufflator (185), or by creating a dust storm in an enclosed cage with a vacuum duster (55).

Schroeder & Mitchell (183) used dusts to mass-mark millions of sterilized tephritid fruit flies. Fruit fly pupae were "tumbled" with dust just prior to adult emergence. The dust was contacted by the expanded ptilinum at emergence and retained in the face of the adult fly. Most of the dust adhering to the body of an emerging fly is quickly groomed off, but the dust enclosed in the ptilinum remains (Figure 2). The dust particles enclosed in the ptilinum are often not visible to the naked eye. In such cases, most marked flies are detected with a UV light. Faintly dyed flies can be detected by dissecting or crushing the head with a blunt object onto filter paper and observing it under UV light and a dissecting microscope (56).

Self-marking techniques were developed for mark-capture studies in which dusts were placed strategically near insect nest and hive entrances (52, 151), insect floral-visitation sites (151, 185), insect bait stations, and insect traps (68, 92, 106). For example, honeybees have been directly marked in the field after they walked over fluorescent dust placed at their hive entrance (151). Price & Slosser (173) placed fluorescent dust in pheromone-baited traps modified to allow for the escape of trapped boll weevils exposed to the dust. Adult bark beetles (*Dendroctonus frontalis* and *Ips grandicollis*) were self-marked as they emerged from logs that were treated with dust (40). Insect self-marking techniques that use dusts are advantageous because they eliminate the damage associated with handling under artificial conditions.

Dusts are most frequently used for marking insects for conventional MRR studies. Field-collected or laboratory-reared insects are dusted in mass and released into the field for dispersal studies (183, 199). However, dusts have also been used to mark insects for mark-capture studies by direct application in the field (30, 171).

Dusts are excellent markers for most insects because they are inexpensive, readily available, environmentally safe, and easily applied and detected. Moreover, different colors of dusts can be used to mark different cohorts of individuals. This characteristic is advantageous when different groups of insects have to be marked

for investigations of intercrop dispersal, colonization rates, and habitat selection (23, 106, 199).

Dusts are often detectable by direct visual inspection (186); however, sometimes a microscope can be helpful for detecting minute quantities of dust on insects. The detection of fluorescent dusts can also be significantly increased under UV light (56, 153, 198, 199, 208, 216).

There are a few drawbacks to using dusts to mark insects. If too much dust is applied, it can kill the insect or produce adverse behavioral effects. Therefore, preliminary tests are needed to find the optimal amount of dust to mark the insects without causing adverse effects. Dusts were reported to inhibit normal dispersal behavior (35) and decrease insect longevity (146, 176, 187). Often dusts are not persistent enough for long-term studies. Another potential drawback to using dusts is that the dust particles can be transferred to unmarked insects in the field or in traps and sweep nets used for sampling (149).

Dye Marking

Various dyes have been used to internally mark insects. Most of the progress toward marking insects with dyes was made in the 1960s and 1970s with the concurrent development of sterile insect release (SIR) and area-wide pest management programs (66, 100, 184, 196). A key to the success of these programs was having an inexpensive and reliable mark that could be easily applied and detected on millions of laboratory-reared insects.

Researchers found that certain oil-soluble dyes accumulate in insect body fluids or tissues after insects have eaten them. Gast & Landin (66) examined the feasibility of marking the adult stage of *Anthonomus grandis* by adding 60 oil-soluble dyes to their larval diet. Only Calco red N-1700 produced a highly visible and long-lasting color that did not yield any adverse side effects on boll weevil growth and development (66, 131). Calco red N-1700 accumulated in the fat body of the larvae, pupae, and newly emerged adults and was clearly visible through the integument. Moreover, the dye was transferred to the eggs deposited by marked females. Larvae hatching from the dyed eggs were also marked, but, after the first instar, the color was too faint to be distinguished.

Many lepidopteran pests were marked with various dyes by adding dye directly into larval diets (70, 101). Dozens of dyes were tested for marking the adult stage of *Heliothis virescens* by adding these dyes to their artificial diet (101). Calco red N-1700 was retained in the integument, fat body, and ovaries of the adult moths. The eggs laid by marked females were also marked, but the larvae that emerged from these eggs were unmarked. Other oil-soluble dyes such as deep-black BB, oil-soluble blue II, and rhodamine B were successfully added to larval diets for marking adult moths (100, 102, 216).

Dyes added to the artificial diets of sterilized adult fruit flies have also proven to be useful markers. Sudan deep-black BB turns the hemolymph of the melon fly, *Dacus* (=*Bactrocera*) *cucurbitae*, black without affecting its growth and

development (184). However, Sudan deep-black BB is not a suitable marker for closely related Mediterranean (*Ceratitis capitata*) and oriental (*Bactrocera dorsalis*) fruit flies (183). Instead, calco blue and tinopal were used for marking various fruit fly species (183). However, the detection of each dye is different. For detecting Sudan deep-black BB in a fruit fly, the fly must be dipped in acetone and crushed on filter paper. Conversely, for detecting calco blue, it is critical that the fly is not dipped in a solvent before crushing. For some dyes, such as the fluorescent dye tinopal, the crushed fly must be examined under UV light (183).

Internal dyes are used in MRR studies to investigate various aspects of termite ecology (75, 127, 206). Typically, field-collected termites are placed on filter paper or paper toweling that has been stained with an oil-soluble dye. After a few days of feeding on the stained paper, the termites become internally marked. Marked individuals can then be released and recaptured for studies of termite dispersal, nestmate behavior, and territoriality between nests (110, 119, 207).

Some termite studies require several different colored dyes that are retained in termites for a long time, whereas others require dyes with a short retention interval. Neutral red and Nile blue A have proven useful for long-term studies (11 and 15 weeks, respectively) for certain termite species, but not for others (75, 157, 205, 206). Dyes with short retention intervals do not confound studies in which previously marked specimens have been released. For example, Sudan red 7B was chosen to mark *Heterotermes aureus* for studies of territorial boundaries and antagonistic behavior between colonies because it is retained in these termites for only 11 days after marking (119). The short retention interval for this dye was ideal because releases of marked termites were at least 1 month apart, which was sufficient time for the dye to fade from previously released individuals.

A critical factor when selecting a dye for certain types of termite studies is to ensure that the internal dye does not laterally transfer to nestmates via their social interactions (e.g. trophallaxis). The lateral transfer of a dye from a marked to an unmarked nestmate leads to erroneous estimations of dispersal and population sizes of termite colonies (69, 75).

Internal dyes are typically not effective markers for small parasitoids. However, adults of some wasp species were marked successfully with acridine orange added to their honey diets in laboratory studies. The dye was retained throughout the adult life stage in many but not all of the species examined. Species that retained the dye also transferred it to the egg stage (204). Further testing is needed to determine the feasibility of using acridine orange as a marker for small parasitoids.

Insects can occasionally be self-marked directly in the field by adding a dye to their bait. For example, the efficacy of various cottonseed oil bait formulations used for boll weevil control was tested by adding calco red to their bait (134). This self-marking technique was useful for determining the proportion of field- collected weevils that were attracted to and fed on the various bait formulations. Several different colored dyes were added to peanut butter baits to study the territorial behavior of the black imported fire ant *Solenopis richteri* (228). The ants at each mound were offered different colored bait. Ants were collected, poured onto a

sheet of white paper, and crushed with a hand roller. The paper was then examined under a fluorescent lamp for the presence of the various colored dyes.

Marking insects by incorporating a dye into their diet has many advantages over other marking procedures. First, most oil-soluble dyes are inexpensive and require minimal additional labor for marking because the dyes are usually mixed in oil (e.g. cottonseed oil, corn oil, etc) and then added directly to the diet. Second, incorporating a dye into an insect diet is a self-marking procedure that avoids extraneous handling of insects. Finally, many dyes can be rapidly and nondestructively detected visually in recaptured insects (70). However, some dyes are not visible by direct inspection; therefore, insects must be crushed on filter paper (41, 189) or ground in solvent (e.g. acetone), followed by visual or spectroscopic inspection (6).

Only a few of the dozens of dyes examined as potential insect markers have proven to be effective. Most dyes have too short a retention interval or are harmful to insects (66, 152, 159, 205, 227).

Pollen Marking

Pollen can be used as a self-marking material for mark-capture studies of insect migration, host plant visitation, and host plant feeding (19, 53, 94). Pollen is an outstanding natural marking material for three reasons. First, plants that depend on insects for pollination have evolved to produce pollen that naturally adheres to insect surfaces. Second, the rigid exterior of pollen is composed of one of nature's most enduring protein materials (pollenium). Third, pollen grains are distinctive and can often be identified to genus (118, 120). Furthermore, the distribution and flowering periods of most plants are well known, which helps to establish the origin of captured insects (103).

Nectar- and pollen-feeding insects have often been examined for the presence of specific pollen types. Mikkola (148) suggested that pollen attached to an insect's surface could be used to provide circumstantial evidence of migratory patterns. However, to be an effective marker, the pollen source must be geographically remote from the areas in which pollen-bearing migrants are caught. In a study conducted in Arkansas, 68% of the *Helicoverpa zea* moths collected in pheromone traps possessed pollen from false mesquite (*Calliandra* spp.) or ape's earring (*Pithecellobium* spp.) (103). The closest location of these plants to the trapping sites was in Texas, which suggests that these moths must have migrated $\geq$750 km. Subsequently, the identification of remote pollen types has proved to be a useful technique for also identifying the migration patterns of many other Lepidoptera pests (94, 103, 104, 132, 133).

Pollen identification has been used to gauge the breadth of an insect's diet. For example, gut dissections of boll weevils contained pollen grains of over a dozen plant families (19, 32), indicating that boll weevils have a much wider host range than previously believed.

Pollen identification is achieved with the aid of various methods of light microscopy and scanning electron microscopy (SEM) (28, 53, 118, 214). Light

microscopy, although sometimes effective, requires extensive sample preparation [e.g. sonication, acetolysis, and staining (28, 118)], which is often tedious and destructive to pollen integrity and produces confusing contaminants such as insect lipids and chitinous materials. In contrast, SEM permits direct viewing of the attached pollen grains with more detail, higher resolution, and better depth of field than light microscopy (28, 214). However, SEM is more costly and time-consuming than light microscopy (45, 214).

Mark-capture studies that use pollen as a natural marker have many advantages over conventional MRR techniques. Studies of long-range migration using MRR techniques (e.g. dyes, dusts, genetic markers, etc) are usually impractical because marking insects may be difficult, and recapture rates are usually unacceptably low despite daily releases of hundreds of thousands of insects (103). Moreover, laboratory-reared insects may have behavioral or physiological characteristics that affect their dispersal ability. Finally, studies in which insects are self-marked with pollen eliminate the need for handling the insects to apply other types of markers.

Despite the advantages of using pollen as a natural biological marker for mark-capture studies, this approach has received limited attention in the study of migratory and feeding activities of insects (45, 125, 148). Several factors limit its use for wide-scale application. As mentioned above, an effective pollen marker must be geographically remote from the areas in which the pollen-bearing insects are caught. Additionally, pollen analysis is costly, time-consuming, tedious, and requires expertise in pollen taxonomy (118). Furthermore, the practicality of using pollen as a biological marker can be influenced by the time of the year in which the study is conducted (104, 132).

Genetic Marking

Visible Genetic Markers Visible genetic mutations of laboratory-reared insects have been used to identify insects for MRR studies (11, 15, 57, 182). Visible mutations, which sometimes occur naturally in laboratory cultures, can be found by careful observation. In some instances, mutations are induced by exposing insects to ionizing radiation or mutagenic chemicals (11).

Body and eye colors are the most common and conspicuous visible markers (Figure 3), but more subtle mutations also occur. For example, some genetic mutations include the presence or absence of spots, bands, body parts, hairs, and spines on insects (11). Mosquito dispersal was studied using various visible genetic markers (see 185). Some of the visible mutant traits used for identifying laboratory-reared mosquitoes include *spotted* abdomens, *silver* mesonotums, *bronzed* tarsi, and *black* palp (24, 57, 95). An *ebony* body color mutation was discovered in *A. grandis* (9). This codominant phenotype, which persisted over nine generations in the laboratory and in field cages, was useful for studying boll weevil dispersal and mating behavior (16). In an MRR study, *ebony* weevils were collected up to 2 miles from their release site. Likewise, a dominant

mutation, *sooty*, in *Pectinophora gossypiella*, was able to compete with the feral population and persisted over a full cotton-growing season (14).

Inheritance of genetic markers may be dominant, codominant, recessive, polygenic (controlled by more than one locus), or sex linked. Dominant mutations require only one allele (i.e. are heterozygous) for the phenotype to be present, so the F_1 progeny resulting from matings between the released mutants and feral insects also possess the mark. Therefore, these mutations are useful for multigenerational studies. Codominant mutations, considered by some to be the most useful markers (11), show one phenotype when heterozygous and a different phenotype when homozygous. For example, a heterozygous mutation that produced a *black* body in *Trichoplusia ni* was used as a marker for studies of their population dynamics (13). However, this mutation was lethal in its homozygous form. Recessive mutations, which are exposed only in homozygotes, are useful for studies of a single generation because the mutation disappears in the F_1 progeny after mating with feral individuals. Sex-linked mutations are commonly used for genetic sexing (121, 142, 180) or for sex-linked lethal mutations (12 and references therein). An overview of genetic mutations, including systematic procedures for recovering mutations, is provided by Bartlett (11).

Visible genetic mutations are effective insect markers once a mutation is found or induced because there is little additional cost beyond the maintenance of the laboratory culture. Furthermore, the detection of the marker is by nondestructive visual observation either with the naked eye or with the assistance of a dissecting microscope. Moreover, very little training is required for observers to recognize the marked insects, and in most instances, marked insects can be recognized in the field. A major advantage of visible genetic markers is that they are part of the insects' physical makeup and persist throughout their life spans.

Although visible mutations have been found or induced in insects (15, 123, 179, 182), relatively few have been used as markers in actual field situations, because there are many drawbacks to using visible genetic markers for MRR studies. First, the markers are rare and usually associated only with insects that have been reared for many continuous generations in the laboratory. Second, the fitness of laboratory-reared mutants must be carefully examined to ensure that the mutation does not have any deleterious effects on their physiology or behavior. Third, insect mutations induced by radiation or mutagenic chemicals may include other nonvisible but detrimental mutations. Some of the deleterious effects may be difficult to determine and may require several generations of inbreeding to eliminate (11). Fourth, the type of inheritance of the mutant genotype must be determined and considered in relation to the goals of the experiment. Finally, genetically marked mutants are not practical for mark-capture studies because they must be reared in the laboratory.

Biochemical Genetic Markers Genetic variation between insect races and biotypes occurs naturally within insect species as a result of geographical isolation or host plant use (43). These genetic variations may be useful insect markers. For

example, insect-specific enzymes can identify distinct insect populations. These enzyme "fingerprints" are invisible to the naked eye, so their banding patterns must be detected by various techniques, including polyacrylamide and starch gel electrophoresis. Several authors have reviewed these techniques and the uses of isozymes in studies of population dynamics in insects (10, 112, 163, 197, 202).

Biochemical genetic markers are useful in MRR studies because they do not change the appearance of the insects while providing a measure of both physical dispersal and gene flow (163). Uses for biochemical markers include studying insect dispersal, mating, immigrant origin, population relationships, and insecticide resistance (111, 141, 221).

The major drawback of biochemical genetic markers is that the electrophoretic detection of insect-specific enzymes requires extensive preparation before field studies (163, 202). The fact that there is variation within insect populations requires a great deal of laboratory experimentation to find unique enzyme-banding patterns between populations (15). Once a specific marker enzyme(s) is discovered, the analysis of insect samples is costly, time-consuming, tedious, and potentially subjective. Furthermore, the analysis of insects for specific enzyme patterns by electrophoresis is destructive and impractical for direct field examination.

Radioactive-Isotope Marking

Labeling insects with radioactive isotopes was a popular insect-marking method from the 1950s to the 1970s. However, stricter environmental protection laws coupled with the development of simpler, less expensive, and more reliable methods have reduced the usefulness of these isotopes as insect markers. For these reasons we refer our readers to the outstanding review of radioactive-isotope marking provided by Service (185).

Elemental Marking

Rare- or trace-element-marking techniques were developed in the 1970s as an alternative to marking insects with radioactive isotopes (20, 200). The use of trace elements to mark insects was reviewed by Akey et al (3). Elements were used successfully to mark at least 8 orders and 30 families of insects, ticks, and spiders. Some of the trace elements used for marking insects include the elements rubidium (Rb), strontium, cesium, manganese, hafnium, and iridium and the lanthanide elements lanthanium, samarium, europium, dysprosium, and cerium. Of these, Rb in its chloride form (RbCl) is the most frequently used trace element marker for insects.

Wide varieties of techniques have been used to mark insects with trace elements. An easy and effective method to externally mark insects for MRR studies is to dip the pupae (87) or topically spray the adults in confined laboratory areas (124). The technique used most commonly for elemental marking of laboratory-reared or field-captured insects is to mark them internally by feeding them artificial diets containing a trace element (2, 7, 38, 96, 98, 109, 117, 126, 165, 220). Mosquito

larvae were marked by adding RbCl to the water in which they were reared (192), and tsetse flies were labeled by adding europium and dysprosium to their blood meal (47, 87).

Many self-marking techniques have been developed for labeling insects with trace elements. Imported fire ants, *S. invicta*, were marked after feeding on baits containing a trace element (190). Artificial nectars containing Rb, strategically placed near a light source in the field, were useful for self-marking adult bollworms (46). Sand and silicone gel pupation media containing dysprosium or samarium were successfully used to label emerging adults of *Rhagoletis* sp. in the laboratory (86). Arthropods, including mosquitoes, biting midges, and ticks, were marked after feeding on hosts injected with a trace element (5, 29, 107, 122). The trace element was incorporated into the host's bloodstream and then accumulated in the arthropod as it fed on its host. The use of two different elements was useful for determining whether mosquitoes take multiple blood meals (5).

For mark-capture studies, phytophagous insects were marked with various trace elements, including Rb, cesium, and strontium by feeding on marked host plants. In the method developed by Berry et al (20), foliage was sprayed with an aqueous solution containing the trace elements. In turn, herbivores that fed on marked plants accumulated detectable quantities of the trace elements. This is still the easiest and most commonly used method to mark host plants in the field (3, 7, 42, 58, 74, 165, 168, 194, 210).

Growing host plants in a hydroponic nutrient solution containing a trace element (63) or irrigating plants with a solution containing a trace element (58, 74, 194) are methods unique to marking with trace elements. The elements Rb, cesium, and strontium are absorbed and translocated in plants (129). Marking of plants by the translocation of these trace elements sprayed on the foliage or supplied in nutrient or irrigation solution is useful for marking both chewing and piercing-sucking herbivores.

Many other innovative techniques have been developed for marking insect host plants. Corn seedlings have been marked by dipping seeds in a solution containing water or milk, Elmer's glue (Elmer's Products, Inc., Columbus, OH), and RbCl, or by planting seeds on paraffin cubes containing RbCl (36, 128). Trees have been labeled by pressure-injecting a solution of RbCl directly into the trunk (26, 145, 164) or by using a passive-stem-well infusion technique (212).

The practicality of marking insects with trace elements is dependent on a variety of factors. (*a*) Trace elements are distributed in small quantities in the Earth's crust. Consequently, their natural levels may vary by geographical location and affect the background levels in plants and insects (20). In some regions where the indigenous level of a given trace element is high, it is difficult to mark insects with enough trace element to separate them from native insects (1, 97). (*b*) The uptake of elements varies among species of plants and insects (62, 165, 217). Therefore, it is crucial that the natural background levels of trace elements be determined for plants and insects of interest at the study site before initiating studies using elemental markers. (*c*) Large insects contain more of the marker than small insects,

adding to the variability between specimens (61). However, many small insects were successfully marked with trace elements, including aphids (74), leafhoppers (162), mosquitoes (5, 108, 122, 192), and whiteflies (44). (*d*) The concentration of the element applied to an insect has a direct linear relationship with the amount of the element retained by the insect (20). It is important to apply the highest concentration possible that does not adversely affect the insect's physiology or behavior but that consistently provides a mark that exceeds the amount in unmarked insects (3). (*e*) The technique used to apply a trace element to an insect can determine the intensity and duration of the mark (65, 97). (*f*) The amount of trace element retained by an insect can decline over time owing to physiological and behavioral factors such as feeding, excretion, mating, and oviposition (62).

Marking with trace elements has many advantages over other insect-marking procedures. Elemental markers are not radioactive, so they are safe for workers and for the environment. There are no tags, paints, dyes, dusts, or visible marks left on insects marked with an element to alter their behavior or interactions with other insects.

Trace elements are retained well during nonfeeding, overwintering stages of insect lives. For example, pink bollworm pupae labeled with Rb were still labeled when the moths emerged in the spring of the following year (219), and boll weevils that had been fed on labeled plants in the fall were still labeled the next spring (232). Emerging parasitoids that overwintered in marked eggs of the prune leafhopper *Edwardsiana prunicola* contained detectable levels of RbCl in their system the following summer (42).

Trace elements are useful for multistage, multigenerational, and multitrophic marking (96). Some immature insects marked with a trace element retain the element throughout their subsequent life stages. Labeled adults of some insect species deposit marked eggs, but the mark rapidly declines from larvae that hatch from labeled eggs (96, 126).

Trace elements are useful for marking some insects for mating studies. For example, pink bollworm, tobacco budworm, and beet armyworm males marked by feeding on a diet containing RbCl transferred the mark via their spermatophores to unmarked females during mating (71, 96, 218).

Trace elements are among the few markers that are useful for marking small parasitoids. Parasitoids that develop in marked hosts are often labeled with trace elements upon emergence from their hosts (42, 58, 115, 212). For example, *Anaphes iole* was successfully marked with Rb when reared from eggs of *Lygus hesperus* adults fed on a diet containing RbCl (114). In a similar experiment, *Microplitis croceipes* was marked by rearing it in *H. virescens* larvae that fed on an artificial diet containing one or more of four trace elements (109).

In addition to the transfer of trace elements from hosts to parasitoids, it has been demonstrated that trace elements can transfer from prey to predators (72). For example, adult *Carabus nemoralis* accumulated detectable levels of Rb after feeding on marked gypsy moth larvae (117).

A limitation to the wide-scale use of trace elements as insect markers in large fields is that the detection of elements can be difficult, expensive, and time-consuming. Analysis of trace elements requires technical expertise and expensive detection equipment (2). Moreover, the lanthanoid series elements are expensive and require the availability of a neutron source to activate the samples.

Some trace elements are not retained very well in certain insect species. It was shown that Rb retention declines rapidly after removal of insects from the Rb-enriched diet or host, especially in the actively feeding stages of insects. For example, Rb could be detected for only 2–6 days after marking aphids (74) and adult *Lygus lineolaris* (62).

High concentrations of trace elements can adversely affect development, survival, and fecundity of certain insects (220). For example, *A. iole* that developed in eggs of *L. hesperus* reared on a diet containing 1000 ppm of RbCl had a shorter life span and lower fecundity than those reared on 500 ppm of RbCl (114). Increased mortality was seen in Rb-marked *H. zea* (46), whereas increased larval developmental times were shown in *T. ni* (201) and *Platynota idaeusalis* (126) with increasing levels of RbCl. Other adverse effects include increased adult deformity and reduced pupation, eclosion, and egg production (96, 126, 201).

Nitrogen-15 Marking Nitrogen-15 (^{15}N) is a trace element that is absorbed and translocated in plants in the same manner as common nitrogen (^{14}N). ^{15}N is a possible alternative to conventional elemental marking (105, 160, 209). ^{15}N was used to mark host plants by adding it to a fertilizer solution. Adult *Cotesia plutellae* and *Hippodamia convergens* that foraged at the flowers of ^{15}N-marked plants showed detectable quantities of the marker. Plant material enriched with ^{15}N was mixed into the artificial diet of navel orangeworms (*Amyelois transitella*). Both the orangeworms that fed on the artificial diet and wasps (*Goniozus legneri*) that parasitized them contained detectable levels of ^{15}N in their systems (195).

The major drawback to marking insects with enriched ^{15}N is that the detection of the marker requires mass spectrometry. Mass spectrometers are expensive to buy and operate, require technical expertise to run, and the analyses of the samples are costly, time-consuming, and tedious. Additionally, mass spectrometer analyses require a relatively large amount of biomass. Small insects, such as *G. legneri*, must be pooled, which precludes the analysis of individual insects.

RECENT DEVELOPMENTS IN INSECT MARKING

Protein Marking

Protein-based methods for marking insects have been developed recently that overcome some of the drawbacks associated with conventional marking techniques. Insects were marked with vertebrate-specific proteins and then examined for the presence of the protein by sandwich enzyme-linked immunosorbent assay

(ELISA), using vertebrate-specific antibodies (77, 78, 80, 83). *L. hesperus* was the first insect marked with a vertebrate protein, rabbit immunoglobulin G (IgG) (80). This technique was developed as a spin-off of established predator gut content ELISAs to distinguish released predators from native ones, while simultaneously analyzing their gut contents by ELISA for the presence of prey remains by using pest-specific monoclonal antibodies (81). Combining predator gut content ELISAs with protein-marking ELISAs proved useful for comparing the efficacy of augmented predator populations and native populations and for examining the dispersal of released biological control agents (84, 85).

Insects can be marked with protein in a variety of ways. For robust insects, the lyophilized protein is simply dissolved in water and topically sprayed on the insects with any common spraying device (77). A perfume atomizer was effective for marking relatively large parasitoids, but was ineffective for marking extremely small and delicate ones. We used a nebulizer to apply protein to the extremely small and delicate parasitoids. A nebulizer is an inexpensive medical device that produces a fine, evenly distributed mist that does not appear to harm or kill parasitoids (78).

The adult stage of *A. iole* (83) and *Trichogrammatoidea bactrae* (78), as well as the adult stage of *Chelonus curvimaculatus*, *Encarsia formosa*, and *Eretmocerus emiratus* (JR Hagler, unpublished observations), were marked with protein externally by contact exposure or topical mist and internally by feeding them a honey solution containing rabbit IgG. Results showed that the rabbit protein was retained throughout the entire adult life span in or on almost every species examined, regardless of the application method. Parasitoids that were internally marked after eating protein-rich honey appeared to slowly digest or excrete the protein, as the ELISA reaction decreased over time. However, the ELISA procedure was able to detect minute quantities of protein for over a week after the parasitoids fed on the labeled diet (83).

Parasitoids were indirectly marked by applying protein to their host just before their emergence from the host. Unfortunately, only 11.2% of the *T. bactrae* emerging from marked pink bollworm eggs and 50% of the *E. emiratus* emerging from marked whitefly nymphs contained detectable traces of rabbit protein (JR Hagler, unpublished observations). The emerging parasitoids did not ingest enough of the marking protein, as they chewed out of their marked hosts, to be detected by ELISA on a consistent basis. Further studies are needed to determine whether the proportion of parasitoids emerging from the marked host can be increased if the host is marked with a more concentrated protein solution.

Protein marking was effective for externally and internally marking several small parasitoid species. This relatively new marking procedure gives researchers another useful tool for MRR studies involving small parasitoids. Protein marking offers many advantages over the other methods used to mark small insects. For instance, both the protein marker and the immunoreagents needed for the ELISA are relatively inexpensive and readily available from immunoreagent distributors. Additionally, thousands of individuals can be marked for MRR studies within minutes by using small amounts of protein. Field tests indicate that verte-

brate proteins are persistent, photostable, heat-tolerant, and water-resistant (77). Perhaps the greatest advantage of protein markers is that the ELISA analysis is simple, rapid, sensitive, and safe. Moreover, the laboratory equipment needed to conduct the analyses is relatively inexpensive and readily available.

Protein markers may be used to study trophic-level interactions. Certain predators that ate *P. gossypiella* eggs and *Bemisia argentifolii* adults externally marked with rabbit IgG tested positive by ELISA for the presence of the marker (82). Results indicate that marking prey externally with protein could be useful for analyzing the gut contents of predators with chewing mouth parts, but not for those predators with piercing-sucking mouth parts. However, in a recent study, most of the *Orius tristicolor*, a piercing-sucking predator that feeds on a single parasitoid, *Eretmocerus mundus*, containing an internal-protein marker, yielded a positive ELISA response for the protein marker (JR Hagler, unpublished observations). Prey marking with proteins gives researchers another indirect method to examine insect predation, and it circumvents some of the drawbacks associated with the development of pest-specific immunoassays, electrophoretic assays, and assays developed to detect radioactive isotopes on marked prey. Unfortunately, the prey must be handled before predator evaluation, a drawback that is shared with the other current prey-marking techniques (8, 139, 140).

Protein marking was used to investigate the flow of incoming nectar in *A. mellifera* colonies by feeding a sucrose (nectar) solution labeled with rabbit IgG to foraging honey bees (51). Worker bees, nurse bees, larvae, and their stored food reserves were collected over time and assayed by ELISA. The protein was an ideal marker for studying nectar flow in a hive because the protein readily transferred via trophallaxis to nestmates. The technique is less costly, less time-consuming, more sensitive, and safer than using radioactive isotopes, which had been used in previous studies (155).

Protein-specific antibodies facilitate the marking of different cohorts of individuals by using different proteins. We determined that anti-rabbit IgG does not react with chicken IgG and vice versa (77). This attribute makes it feasible to mark different groups of individuals with different proteins. This multiple marking approach proved useful for measuring the intercrop movement of *H. convergens* (85) and *E. emiratus* (JR Hagler, unpublished observations).

Using proteins to mark insects is a relatively new procedure. As a consequence, numerous studies still need to be conducted to establish the validity and limitations of this technique. Further studies on the movement of protein labels through the food web are needed. We demonstrated that a specific protein can be used to label insect prey for predation studies (82). However, we did not investigate tritrophic interactions using this technique. Additionally, it is unclear whether host plants can be effectively marked with proteins. It is unlikely that vertebrate proteins will be absorbed or that they will translocate in the plant's tissue like many of the trace elements do (20, 129, 200). However, herbivores (especially chewing herbivores) that feed on plants topically marked with protein should pick up detectable traces of the protein.

The potentially sublethal effects of protein marking on insects have not yet been examined. We note that protein markers do not appear to possess any adverse side-effects on insects (77, 78); however, studies are under way to determine whether they affect insect physiology and behavior.

Retention of protein markers on insects maturing from one life stage to another is variable. Preliminary studies indicate that first-instar pink bollworms that fed on an artificial diet containing rabbit IgG retained detectable traces of protein throughout their larval and pupal stages. However, the protein mark was undetected in the adult stage (JR Hagler, unpublished observations). Furthermore, first- or second-instar *L. hesperus* that fed on a protein-rich artificial diet were easily detected for rabbit protein, but the protein was not consistently retained in *L. hesperus* after removal from the protein source and as they matured (CG Jackson, unpublished observations).

Another area for future investigation is to identify less expensive proteins for marking insects directly in the field for mark-capture studies. Previous investigators have marked insects in the field by spraying aqueous solutions of Rb directly over plant foliage or by dusting plant foliage with Day-Glo dust (see above). Although vertebrate proteins are ideal for marking large numbers of insects in a confined space for MRR studies by incorporating protein in diet or topical sprays in an enclosed area, the high cost for large quantities makes them impractical for large-field application for mark-capture studies. Perhaps an inexpensive protein that has a marketed antibody can be substituted for the expensive IgG proteins.

We previously showed that the sandwich ELISA format was superior to the direct ELISA format for identifying protein on adult *H. convergens* (77). However, a dot blot immunoassay, an indirect ELISA, or a Western blot assay might be a useful immunoassay format under certain circumstances (73, 79).

Genetically Engineered Marking

Gene transfer systems that use transposable elements such as *P, hobo, Hermes, mariner, Minos*, and *piggyBac* were successfully harnessed as gene vectors to achieve genetic transformations in insects for a variety of reasons (e.g. lethal genes, genetic sexing, genetic sterility, etc) (22, 37, 90, 116, 130, 135, 136, 156, 211). Gene transfer using transposable elements may be used to permanently mark mass-reared insects for SIR programs. For example, the *piggyBac* transposable element containing the *white-eyed* mutant gene of the Mediterranean fruit fly, *C. capitata*, was used to transform *C. capitata* and *D. melanogaster*. The resulting transformations yielded a certain percentage of flies possessing highly visible "white eyes" that remained stable for 15 generations (88, 89).

Effective gene transfer systems could facilitate the creation of insect strains with visible external markers useful for SIR programs. However, there are many difficulties to applying this technology for wide-scale use. First, transposable elements have been applied successfully to only a narrow range of insects, including a few

species of dipterans and lepidopterans (211). Second, germline transformations relying on mutant markers are rare; therefore, mutant recipient strains and cloned copies of the mutant gene are often unavailable (167). Third, ethical considerations need to be thoroughly examined before developing and releasing genetically altered insects into the environment. Fourth, the development of the methodology for transforming insects using transposable elements requires an enormous amount of preliminary research and technical expertise.

Transposable elements carrying green fluorescent protein (GFP) were recently used as biological-marker genes in a wide variety of studies. GFP, a protein specific to the jellyfish, *Aequorea victoria*, was identified over a half century ago, but it was studied for years in virtual obscurity (49, 150, 188). An amazing characteristic of GFP is that it fluoresces naturally under UV light. Recently, GFP was cloned (34) and fused with animal and plant proteins and cells to create "glowing" animals and plants. Over the past 5 years, thousands of biomedical researchers have used GFP for in vivo labeling of prokaryotic and eukaryotic cells (33). The visible, genetically engineered flurophore is an invaluable marker for gene expression and protein-targeting studies in biomedical research. Human gene therapy and drug screening are such uses (137, 213). Recently, insect molecular biologists have used GFP to study a wide range of biological functions including sperm competition, egg development, and neurobiology (99, 172, 223).

GFP has received an enormous amount of attention as a biological marker for studying physiological processes in animals and plants (33). However, its physical properties also make it an ideal candidate for marking insects for ecological studies. Recently, pink bollworm was transformed with the *piggyBac* transposable element carrying enhanced GFP (Figure 4). To date, the transformed pink bollworm strain has been shown to segregate in Mendelian ratios for 15 generations under laboratory conditions (166, 167). Ultimately, researchers plan to introduce this marker gene into the pink bollworm colony used for the SIR program in California.

Currently, GFP is the most popular bioluminescent product on the market; however, other natural or synthethic bioluminescent products might also be useful for marking insects for ecological studies. For example, luciferase, the enzyme responsible for giving fireflies their glow, has been cloned and expressed in other organisms including *Escherichia coli* and tobacco plants (39, 161). A red fluorescent protein, unique to the sea anemone relative *Discosoma* sp., has been used in multilabeling experiments with GFP (76, 138). Additionally, several GFP variants have been developed (e.g. red, blue, and yellow) that fluoresce at different wavelengths for use when multiple colors or markers are needed.

Tagging insects with genetically engineered GFP offers several advantages over conventional marking procedures. First, genetically modified organisms (GMOs) containing GFP are permanently marked because the gene is integrated into the insect's genome. Second, depending on the gene, the genetic element that controls expression of GFP can potentially be expressed during one or all life stages. Third, GFP illuminates independently of cofactors or substrates; therefore, GFP

can be expressed in most cases simply by exciting it with the appropriate wavelength of light (99). Fourth, if GFP is introduced into an insect colony and bred to homozygosity, all of the insects would have the desired mark (167). This could be important for SIR programs that currently rely on dye and dust markers. Finally, unlike genetic transformations that require a mutant marker, GFP transformations can be applied to a wide variety of insects.

GFP can be detected in insects by a variety of techniques. In some cases, GFP can be detected using a fluorescence microscope. However, a spectrofluorometer could provide greater sensitivity or faster analyses if GFP is expressed at sufficiently high levels. The detection of GFP in GFP-marked pink bollworm adults can be difficult. Therefore an alternative approach for detecting GFP was developed using polymerase chain reaction (PCR). Amplification of GFP DNA by PCR provides additional confirmation of the presence of GFP in the pink bollworms that might not show GFP by fluorescence microscopy (166).

Marking insects with genetically engineered proteins has enormous potential for future MRR studies. However, many drawbacks must be overcome before they are readily accepted as useful markers. First, as mentioned above, ethical considerations must be addressed before developing additional GMOs. As a consequence, a major barrier to using GFP as a marker is getting regulatory approval to develop GMOs and then getting further approval to release GMOs. Second, the development of genetically marked insects requires preliminary research that is both time-consuming and costly. Therefore, the greatest potential for using GMOs is in large-scale SIR and area-wide pest management programs. Finally, the detection of GFP and similar proteins by fluorescence microscopy is costly as is the detection of GFP DNA by PCR, which is also tedious and time-consuming (166).

CONCLUSIONS

Choosing the best technique for marking insects for recognition in the field is essential to the success of many research projects. Unfortunately, there is no universal insect-marking technique for all insects. Researchers should first consider using the simplest and most effective markers for their studies. Only when the conventional marking techniques are no longer useful should the more difficult techniques be considered. Perhaps the most important consideration when choosing an insect-marking technique is the potential effect of the marker on the insect's biology and behavior. Deciding which marking technique is best may also include a consideration of the size, life stage, and habitat of the marked insect. The potential toxicity of the marker to the insect, duration of the study, cost-effectiveness, and environmental safety of the marker also must be factored into the decision. Additionally, the utility of any given marking technique depends on whether the experiment is an MRR or a mark-capture study. Careful testing of any chosen marking technique on captive insects before field study is highly recommended.

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Figure 1 A Colorado potato beetle marked with punctures [photograph provided courtesy of Tom Unruh with permission granted by *The Canadian Entomologist* (see reference 215)].

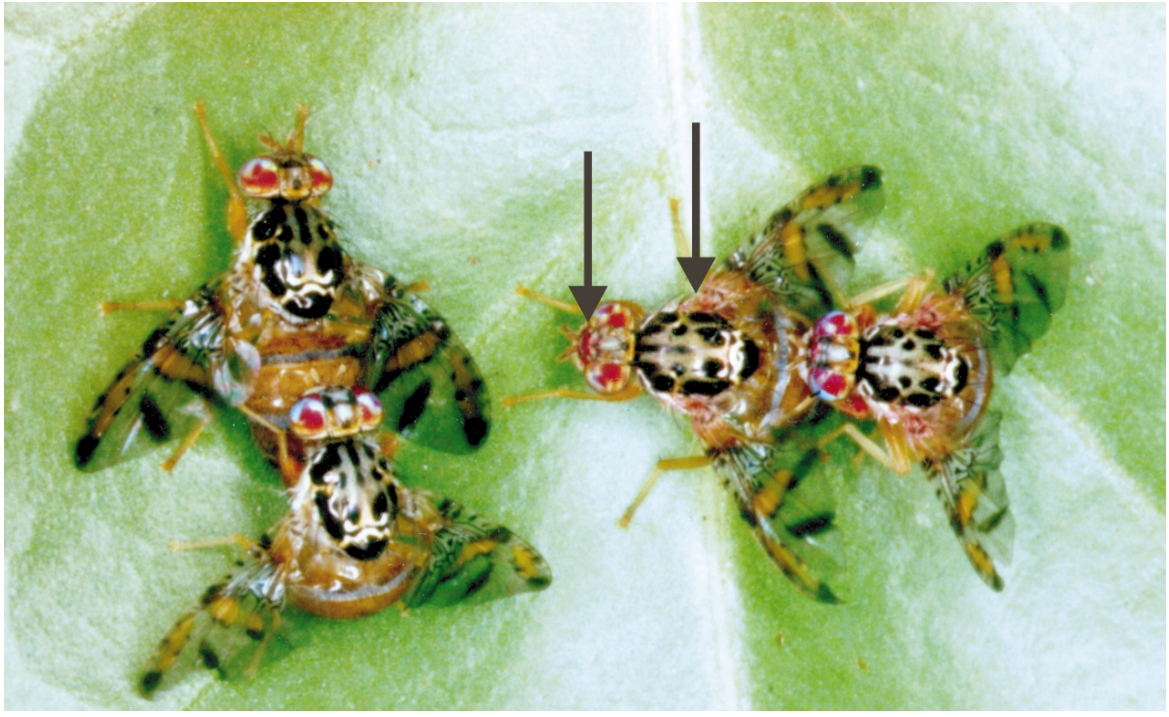

Figure 2 Two mating pairs of Mediterranean fruit flies; one pair is unmarked (left), and the other pair is marked with fluorescent dust as indicated by the arrows (*right*) (photograph provided courtesy of Don McInnis).

Figure 3 Two "red-eyed" mutants and a normal big-eyed bug, *Geocoris punctipes* (insects provided courtesy of Steve Naranjo and photograph provided courtesy of Scott Machtley).

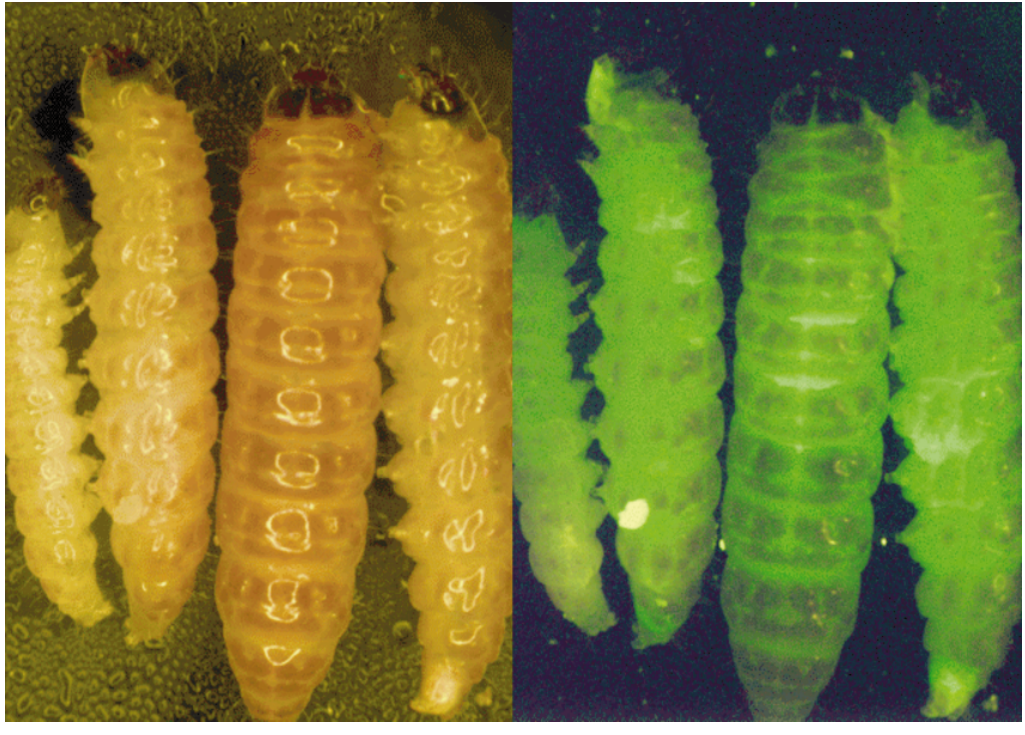

Figure 4 Expression of enhanced green fluorescent protein (EGFP) in genetically modified pink bollworm larvae. The larvae are illuminated under white light (*left*) and under EGFP-excitation wavelength light (*right*) [photograph provided courtesy of John Peloquin with permission granted by *Insect Molecular Biology* (see reference 167)].

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